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Antinutritional and phytochemical composition of fermented condiment *(Ogiri)* made from Sandbox *(Hura crepitan)* Seed

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Abstract

Antinutritional and phytochemical composition of fermented condiment (Ogiri) made from sandbox seed were investigated as affected by the fermentation time. The de-hulled sandbox seeds were washed, wrapped in plantain leaves, boiled for 12 h and fermented at 35°C for 1 to 4 days. The phytochemical screening carried out at different fermentation period using the standard determination process showed a significant decrease in percentage levels of alkaloid from 9.53% to 3.55%. There was a significant reduction in levels of flavonoids from 1.88% to 0.39% indicating over 20.74% decrease due to the fermentation. The saponin levels significantly decreased from 3.07 ± 0.19^{a} to 0.42 ± 0.04^{e} %.The values of tannins ranged from 0.53 to 3.30 mg/kg, while that of Oxalate showed a significant reduction from 5.64 to 4.12 %. The value of the phytates ranged from 1.0 × 10^{-3} to 1.5 × 10^{-2} mg/kg, whereas the cynogenic glycosides' composition in the samples ranged from 7.3 × 10^{-4} to 3.0 × 10^{-4}. Apart from phytates and cyanogenic glycosides, other phytochemicals showed significant ($p<0.05$) differences due to fermentation period.

Keywords: Sandbox Seed (*Hura crepitan*), *ogiri* condiment, phytochemicals, antinutrients, fermentation

1. INTRODUCTION

Phytochemicals which are sometimes regarded as anti-nutritional factors are biochemical compounds present in foods of plant origin, whose intake reduces or impairs proper nutrient utilization in man or animal (Abhishek *et al.*, 2019). Anti-nutritional factors are known to be poisonous to humans when present in different food substances in varying amounts depending on the kind of food. They negatively interfere with nutrient bioavailability by interfering with metabolic processes associated with nutrient absorption in the body (Umaru *et. al*. 2007). Anti-nutritionals factors present in most foods of plant origin include; alkaloids, tannins, phytate, trypsin inhibitor, cyanide, saponins, and oxalates. Phytate and oxalates have the ability to form chelates with most minerals, thereby making them unavailable for absorption by the body. (Wasagu *et al* 2013).

Fermentation being the oldest form biotechnology functions to reduce anti-nutritional properties and produces secondary metabolites through the activities of microorganisms (Paulová, *et al*., 2016). Fermentation increases food safety by reducing toxic compounds present in food materials especially in traditional fermented condiment such as *ogiri* condiment from castor seed (*Ricinus communis*). According Omafuvbe *et al*., (2004), *Ogiri* is a traditional solid state fermented condiment which possesses a characteristic ammoniacal flavor that enhances the taste and flavour of traditional delicacies in Nigeria.

Although *ogiri* provides a non-meat protein substitute and serves as a functional ingredient in Nigerian delicacies, most of the substrates associated with its production are loaded with significant portion of anti-nutrients. Some of these anti-nutritional factors known as secondary metabolites, are active include; saponins, tannins, flavonoids, alkaloids, oxalates, phytates, cyanogenic glycosides.

Sandbox (*Hura crepitans*) seed of *Euphobiaceae* family is native to tropical regions of North and South America. It is also common to

the African continent especially in Nigeria where it is abundant (Oyeleke, *et al*., 2013). According to Gbadamosi and Osungbade (2017), sandbox tree maintains its foliage all through the year. For this reason, it is often planted as a shade tree in the tropics of Africa, of which Nigeria is not exempted. The woody fruits which have similar resemblance with small pumpkin pod, has about thirteen seeds in each pod. Studies on sandbox (*Hura crepitans*) seed revealed contained 25.75% crude protein, 23.52% crude fat and 31.51% carbohydrate (Fowamola and Akindahunsi, 2017). According to studies by Ahaotu *et al*. (2020), cooked unfermented seeds of sandbox contain approximately 19% crude protein, 32.05% fat, 2.37% fibre, 2.88% carbohydrate, 1.81% ash and 41.9% moisture. Moreover, the seed of sandbox is found to be rich in lipid as it is in the same family of *Euphorbiaceae* with castor seed (*Ricinus communis*) and may serve as an important substrate in fermented condiment production, thereby finding important food use for the sandbox seed. There is need to ascertain the quality and edibility of *ogiri* condiment produced from sandbox seed by determining the effect of fermentation on the anti-nutritional properties of sandbox *ogiri* condiment.

2. MATERIALS AND METHODS

2.1. Materials

All the sandbox seeds were obtained from various locations in Imo and Rivers states. All equipment, instruments and analytical grade reagents used for this study were obtained from the University of Port Harcourt, Faculty of Science Research Laboratory, Port-Harcourt Rivers State.

2.2. Sample Preparation and Fermentation

The sandbox seed were sorted and manually de-hulled to obtain the cotyledon which were wrapped in blanched plantain leaves and cooked for 12 h in 2 L of water. The wrapped cooked sample was left to ferment at 35°c for 1 to 4 days. Samples were drawn at different fermentation time of 0, 1, 2, 3 and 4 days and subjected to analysis.

2.3. Qualitative Determination of Chemical Constituents

The determination of the Phytochemicals; Alkaloid, Flavonoid, Saponin, Phytate, Tannin, Oxalate and Cyanogenic glycosides were done through the following procedures:

The qualitative and quantitative analysis of alkaloids, tannins, saponins, flavonoids were determined by using the methods of AOAC (2000).

2.3.1. Determination of alkaloids

Approximately 5 g of the sample was weighed into 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hrs. This was filtered and the extract was concentrated in a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to extract until precipitation occurs. The precipitates were collected and washed with dilute ammonium hydroxide and then filtered. Then the residue was dried and weighed.

$$\text{Calculation; } \frac{W3 - W2}{W1} \times \frac{100}{1}$$

Where; W1 = weight of sample, W2 = weight of empty flask, W3 = weight of flask and residue

2.3.2. Determination of flavonoid

Presence and quantity of flavonoid in the fermented samples were determined using the method of Obadoni and Ochuko (2001) by boiling 5 g of each processed sample in 100 mL of 2 M HCL solution for 40 mins. The hot boiled mixture was allowed to cool to room temperature before being filtered through Whatman filter paper. Flavonoid in the filtrate was precipitated by drop-wise addition of concentrated ethyl acetate until in excess. Following filtration, the flavonoid precipitate recovered was oven-dried and weighed. Percentage flavonoid was obtained by the weight difference of the oven-dried precipitate.

$$\text{Percentage flavonoid} = \frac{W3 - W2}{W1} \times \frac{100}{1}$$

Where; W1 = weight of sample, W2 = weight of empty flask, W3 = weight of flask and residue

2.3.3. Determination of cyanogenic glycosides by ion chromatography

One (1) gram of the sample was weighed into a clean distillation flask, 20 mL distilled water was added and the sample was allowed to stand overnight for proper hydrolysis to be attended. The sample was distilled into 20 mL sodium hydroxide containing 0.5 g crystal. The distillate was titrated with 0.02 N silver nitrate in the presence of 0.2 mL 5% potassium iodide and 1 mL 6 N ammonia hydroxide solution to a permanent turbidity.

Calculation ;

$$1 \text{ mL } 0.02 \text{ N } AgNO_3 = 20 \text{ N or } 1.08 \text{ HCN}$$

$$\% \text{ CN} = \frac{\text{T X } 2/1000}{\text{W1}} \text{ X } \frac{100}{1}$$

Where; T = titre, W1 = weight of sample

2.3.4. Determination of Saponin

Ten (10) mL of 20% aqueous ethanol was added 5 g of the mashed sample, the mixture was put in shaker hot water bath for 4 hours at about 55^0 C. The mixture was filtered and the residue was re-extracted using 20% ethanol. The concentrate was transferred into 250 mL separating funnel and 20 mL of diethyl ether was added and shaken together. The aqueous layer was recovered while the ether was later discarded. The purification process was repeated. 60 ml of n-butanol was added and washed twice with 10 mL of 5% aqueous NaCl (Obadoni and Ochuko 2001).

Saponin content was calculated as:

$$\% \text{ Saponin} = \frac{\text{Initial weight –final weight of the sample}}{\text{Initial weight}} \times 100$$

2.3.5. Determination of phytate by titration

The method of Lucas Markakes (1975) for phytate determination was adopted, where 0.2 g of the sample was weighed into 250 mL conical

flask and soaked in 100 mL of 20 % concentrated HCL for 3 hours. The sample was then filtered and 50mL of the filtrate was introduced into 250 mL beaker then, 100 mL distilled water added. Ten (10) mL of 0.3% ammonium thiocyanate solution was added as indicator and titrated with standard iron (III) chloride solution which contained 0.00195 g iron per 1 ml.

Percentage phytate was calculated as:

$$\frac{\text{titre value} \times 0.00195 \times 1.19 \times 100}{2}$$

2.3.6. Determination of Oxalate

Oxalate Determination by Titration Method

This method of oxalate determination comprises: digestion, oxalate precipitation and permanganate titration.

Digestion

Two (2) g of the sample was suspended in 190 mL of distilled water in a 250 mL volumetric flask, followed by addition of 10 mL of 6M HCL and the suspension digested at 100^0 C for 1 hour. The solution was cooled, and then made up to 250 mL mark before filtration.

Oxalate Precipitation

Duplicate Portions of the filtrate were measured into breakers and four drops of methyl red indicator added. Then NH_4OH solution was added (drop wise) until the test solution changed from pink to faints yellow colour (pH 4.0 - 4.5). Each portion was then heated to 90^0C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was again heated to 90^0C and 10 mL of 5% $CaCl_2$ solution was added with constant stirring. The solution was then heated and left overnight at 25^0 C, after which it was centrifuged at 2500 rpm for 5minutes. The supernatant was decanted, and the precipitate completely dissolved in 10 mL of 20% (v/v) H_2S0_4 solution.

Permanganate Titration

Here the total filtrate resulting from digestion of 2 g of sample was made up to 300 mL. Aliquots of 125 mL of the filtrate was heated until near boiling and then titrated against 0.05 M standardized $KMnO_4$ solutions to a faint pink colour which persisted for 30 seconds. The percentage oxalate was calculated using the formula:

$$\frac{T \times (Vme)\ (Df) \times 100}{(ME) \times (M_F)} \qquad 1$$

Where T is titre of KMnO4 (ml),
Vme is the volume-mass equivalent
Df is the Dilution factor = Vt/A
Where Vt is the total volume of filtrate (300ml) and
A is the aliquot used i.e. 250ml,
ME is the molar equivalent of KMnO4 in oxalate and
Mf is the mass of sample used

2.3.7. Determination of Tannin by method described by (Makkar *et al*., 1993). Tannin was determined by adding 40 mL diethyl ether containing 1% acetic acid (v/v) to 0.4 g of each sample. The mixtures were mixed to remove pigment materials. The supernatant was carefully discarded after 5 mins and 20 mL of 70% aqueous acetone added and the flask sealed with cotton plug covered with aluminum foil, then kept in shaker for 2 hours for extraction. The content of the flask was filtered using Whatman filter paper (No. 2). Aliquot 0.5 mL filtrate was made up to 1.0 mL with distilled water and 0.5 mL Folin Ciocalteu reagent was added to the mixture, then mixed properly before addition of 2.5 ml of 20% sodium carbonate solution and further mixed. The mixtures were kept for 40 mins at room temperature, after which absorbance was taken using spectrophotometer and concentration was estimated from the tannic acid standard curve.

$$\text{Soluble tannins (\%)} = \frac{C\ (mg) \times \text{extract volume (m)}}{10 \times \text{Aliquot (ml)} \times W1\ (g)}$$

2.4. Statistical analysis

Data obtained from the study were subjected to analysis of variance (ANOVA) using a statistical software SPSS version 22.0 and judged significantly at 95% confidence level ($P<0.05$).

3. RESULTS AND DISCUSSION

Table1. The phytochemical and anti-nutrient content of *ogiri* condiment made from sandbox seed as affected by fermentation

Sample	Alkaloids (%)	Flavonoids (%)	Saponins (%)	Tannin (mg/kg)	Oxalate (%)	Phytate (mg/kg)	Cyanogenic Glycosides (mg/kg)
SAA	9.53 ± 0.04^{a}	1.88 ± 0.17^{a}	3.07 ± 0.19^{e}	0.53 ± 0.34^{c}	5.64 ± 0.11^{a}	1.5×10^{-2}	7.3×10^{-4}
SAB	8.40 ± 0.28^{b}	1.32 ± 0.06^{b}	1.49 ± 0.08^{c}	0.38 ± 0.06^{c}	5.00 ± 0.19^{b}	3.0×10^{-3}	7.5×10^{-4}
SAC	6.72 ± 0.01^{c}	1.30 ± 0.00^{b}	2.52 ± 0.04^{b}	0.86 ± 0.01^{b}	4.12 ± 0.19^{c}	1.0×10^{-3}	3.5×10^{-4}
SAD	3.55 ± 0.08^{d}	1.29 ± 0.04^{b}	0.42 ± 0.04^{e}	3.30 ± 0.12^{a}	4.95 ± 0.13^{b}	1.5×10^{-2}	3.5×10^{-4}
SAE	8.48 ± 0.06^{b}	0.39 ± 0.01^{c}	0.95 ± 0.08^{d}	1.15 ± 0.21^{b}	4.71 ± 0.06^{b}	1.5×10^{-2}	3.0×10^{-4}
LSD	**0.35**	**0.21**	**0.27**	**0.29**	**0.37**	-	-

Day 0 (SAA) of fermentation; Day 1 (SAB) of fermentation; Day 2 (SAC) of fermentation; Day 3 (SAD) of fermentation; Day 4 (SAE) of fermentation, Superscript letters: (a, b, c, d, and e) shows the order of significant difference

The result of the phytochemical and anti-nutrient study of *ogiri* condiment made from sandbox seed as affected by fermentation time shown in the table above revealed the presence of phytate, saponins, oxalates, alkaloids, flavonoids, tannin and cyanogenic glycoside. According to Settharaksa *et al*. (2012), the presence of these phytochemicals in foods of plant origin indicates their possible usage in the production of functional foods and use for medicinal purposes. The highest content of anti-nutritional factors in the sandbox *ogiri* condiment detected was in the alkaloids and oxalates, while the least where in the phytates and cyanogenic glycosides contents. Fermentation was found to cause a significant reduction in all the anti-nutritional properties studied, except for tannin which revealed a significant increase. There was no significant difference ($p<0.05$) in the phytate and cyanogenic glycoside content of the study samples.

There was a significant reduction in the percentage level of Alkaloid from the sample as a result of fermentation from 9.53% in SAA to 3.55% in sample SAD. This reduction could be attributed to the activities of fermentative microorganisms associated with fermentation (Fowomola and Akindahunsi, 2008) since a good number of microorganisms has been reported to be involved in the natural

fermentation of *Hura crepitans* seeds (Fagbemi and Atum, 2001). A significant increase was also observed in alkaloid content of sample SAE. Alkaloids are bitter-tasting compounds which are mostly toxic and widespread in nature, found in many plants, especially medicinal plants (Awuchi, 2019).

In this study, the study samples which represents samples fermented at different days revealed a significant ($p<0.05$) reduction in percentage concentration of flavonoid from 1.88 % in sample SAA (unfermented) to 0.39 % in sample SAE (fourth day ferment), indicating over 20.74% decrease in the level of flavonoid due to the fermentation. Adebayo *et al*. (2019) also reavealed a reduction in flavonoids in samples of solid state fermented castor bean seeds using *Pleurotus ostreatus*. This could be attributed to the activities of microbial enzymes produced during fermentation which are able to hydrolyze and break down plant cell walls, leading to the reduction in flavonoids (Hur, *et al.* 2014). Also according to Nazarni, *et al*. (2015), fermentation results to the production of microbial ß-glycosidase, which hydrolyze phenolics and flavonoids leading to the reduction of flavonoid. Flavonoids are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage and having strong anti-carcinogen activity.

The percentage saponin concentration revealed a fluctuational significant reduction as a result of fermentation from 3.07 % in sample SAA to 1.49 % in sample SAB, with an increase to 2.52 % in sample SAC, further reduction to 0.42 % in sample SAD and finally to 0.95 % in sample SAE. This result is in agreement with studies by Gbadamosi and Osungbade (2017) who reported that saponins in *Hura crepitans* decreased from 0.18 to 0.10 mg/100 g due to fermentation. Also according to Adebayo (2019), saponin was reduced in '*Ogiri'*, a locally made condiment from castor seeds (CSF), which may be as a result of long boiling time and fermentation which Oranusi, *et al*. (2014), reported to reduce phytochemicals/anti-nutritional concentration in raw food to acceptable nutritional level. Saponins are known to bind cholesterol, thus interfere with cell growth and division (Okwu 2004; Okwu and Emenike, 2006).

Fermentation revealed a fluctuational significant increase in tannin content of the samples. The values of tannins ranged from 0.53

mg/kg in SAA to 3.30 mg/kg in SAD and final reduction to 1.15 mg/kg in sample SAE. This increase in tannin concentration of the fermenting samples may be due to the type of microbes associated with the fermentation which may be lacking the tannase enzyme. This result is in contrast with the studies by Adebayo, *et al.,* (2019) reported tannin decrease as a result of fermentation of castor and watermelon seed respectively.

The samples showed a significant reduction in percentage oxalate from sample SAA to SAC (5.64 to 4.12 %). Sample SAC had the least significant decrease in oxalate. This implies that there is a great reduction in percentage oxalate after day 3 fermentation. This revelation is in line with the study by Gbadamosi and Osungbade (2017), which reported a reduction in oxalate concentration as a result of fermentation. Oxalate reduces calcium availability both in man and in non-ruminants, at higher dose of 1 g to 2 g/kg of body weight, toxic to the kidney and heart (Akpabio, *et al*., 2012). Higher oxalate content contains more than 10 mg per serving, while low content has less than 2 mg per serving. The analysis of variance showed that the oxalate content of the unfermented seed (day 0) vary significantly ($p < 0.05$) from those of the fermented samples at other days.

The value of the phytic acid ranged from 1.0×10^{-3} to 1.5×10^{-2} mg/kg. These values showed no significant difference, although the values were reducing as fermentation time increases. The values obtained in this study are lesser than those reported by Fowomola and Akindahunsi (2008). Phytate and phytic acid have a strong binding affinity to dietary minerals, calcium, zinc, and iron, inhibiting their absorption (Schlemmer, *et al*., 2009).

The cyanogenic glycoside composition of the study samples SAA to SAE were 7.3×10^{-4} to 3.0×10^{-4} mg/kg respectively and it showed no significant difference. Fowomola and Akindahunsi (2008) reported that fermentation significantly reduced the toxic levels of cyanide from 1.66 to 0.6 mg/100 g. These values were slightly higher than the values reported in this study.

4. CONCLUSION

Generally, fermentation of the study samples for *ogiri* condiment production resulted in the significant reduction of the anti-nutritional properties of sandbox within 3-4 days of fermentation, except for tannin which showed a significant increase in its content. Alkaloids decreased significantly as the fermentation days increased and is advised to stop fermentation on the 3rd as it has the least level of Alkaloids. Flavonoids had a significant reduction as the fermentation days increased and it had the lowest level on the 4th day of fermentation and that is acceptable. Saponin, Tannin and Oxalate had fluctuational reduction as the fermentation days increases, although it is safer to end fermentation after four days because it produced samples with lowest level of these phytochemicals.

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